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Effects of Ciprofloxacin and Ofloxacin on Adult Human Cartilage In Vitro

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Chondrocyte toxicity and necrosis were seen with electron microscopy after incubation of human adult cartilage biopsy specimens in ciprofloxacin or ofloxacin. In vitro exposure of chondrocytes to fluoroquinolones did not affect apoptosis as determined by flow cytometry. While the immediate clinical significance of this finding remains unclear, the possibility of long-term cartilage damage after fluoroquinolone treatment cannot be excluded.

The use of fluoroquinolones for children has been critically discussed because of chondrotoxic effects in growing animals, for which irreversible damage to the cartilage of the weight-bearing joints with formation of blisters and erosions and degeneration of the chondrocytes has been observed (13, 15, 27, 28, 31).

We investigated the effect of quinolones in organ cultures of human adult cartilage.

Five-millimeter punch biopsy specimens of full-thickness cartilage were taken from the weight-bearing parts of the condyles of the knees of five male victims of traffic accidents (age range, 22 to 32 years) within 6 h of death. The ethics committee of our hospital had approved the biopsy protocol. Cartilage explants were cultured for 2 weeks in cartilage culture medium either alone (Ham F-12 supplemented with 25 mM HEPES, 1 U of human recombinant insulin per liter, and 20% fetal calf serum) or with the addition of 1 and 10 mg of ciprofloxacin or ofloxacin per liter, respectively. The culture medium was replaced every 3 days. After fixation (2.5% glutaraldehyde in 0.1 M cacodylate buffer [pH 7.4] and 1% osmium tetraoxide), cartilage specimens were dehydrated in ethanol, incubated in propylene oxide, and embedded in Spurr's ultraviscosity resin. Ultrathin sections (60 nm) were viewed with a transmission electron microscope (EM 109; Carl Zeiss, Oberkochen, Germany). Light microscopic images of semithin sections (0.4 μm), stained with toluidine blue, were analyzed with a Vidas image analysis computer (Kontron, Munich, Germany). The evaluation of the cartilage explants referred to the four main zones: the superficial layer, the middle layer, the deep layer, and the calcification zone that is in close proximity to the bone (8). Areas of 200 μm^2 were framed, and the percentage of empty chondrons was determined.

For suspension cultures, chondrocytes were isolated by digestion with 0.5% pronase and 0.08% collagenase type II, adjusted to 5×10^6 cells/ml, and incubated with and without the fluoroquinolones as indicated above for 3, 10, and 21 days. After fixation in paraformaldehyde and permeabilization in 0.1% Triton X-100, nick end labeling with fluorescein isothiocyanate-dUTP was performed (In-Situ cell death kit; Boehringer, Mannheim, Germany). As a positive control for the detection of apoptotic cells, we used blood mononuclear cells from healthy volunteers pretreated with dexamethasone for 24 h. Cytofluorographic analysis of the percentage of cells undergoing programmed cell death was performed with a FACScan cytometer (Becton Dickinson, San Jose, Calif.).

After culture in medium alone, electron microscopy showed active chondrocytes with an abundant rough endoplasmic reticulum, numerous mitochondria, a high number of vacuoles, and distinct deposits of glycogen. The nucleus showed well-developed nucleoli and a scattered distribution of euchromatin (Fig. 1). Necrotic chondrocytes were not observed in the organ cultures without gyrase inhibitors. Culture of human cartilage explants with either ciprofloxacin or ofloxacin led to a significant number of necrotic chondrocytes with an abundant accumulation of glycogen and hypervacuolization (Fig. 2). Necrosis of chondrocytes could be demonstrated mainly in deeper layers of the articular cartilage. The quinolone-treated cartilage explants were characterized by areas of prominent bundles of intermediate filaments (Fig. 2b, insert). In several chondrocytes of zones II and III, these bundles occurred as a perinuclear ring of filaments. A remarkable increase in the number and size of cell surface projections was seen (Fig. 2a). Necrosis of chondrocytes after treatment with ciprofloxacin as well as ofloxacin occurred not only in the calcification zone (IV) but also to a remarkable extent in the other zones of cartilage. This form of necrosis was characterized by a condensation of nuclear chromatin (Fig. 3a). In contrast to culture of adult cartilage explants in medium alone, incubation with fluoroquinolones was associated with the formation of matrix vesicles with enclosed calcium apatite crystals. These microcalcifications and high amounts of proteoglycan aggregates (Fig. 3b) were found not only in zone IV but also in zones II and III.

Comparison of the two quinolones used with regard to the toxic effects seen with electron microscopy showed analogous changes, whereas the difference from cartilage cultured with medium alone was impressive.

On light microscopy, exposure to gyrase inhibitors led to loss of chondrocytes from within the chondrons (Fig. 4). An artifact of empty chondrons due to the processing (as often seen with paraffin sections) can be excluded in this case since the specimens were embedded in epoxy resin. In addition, fragmenta-

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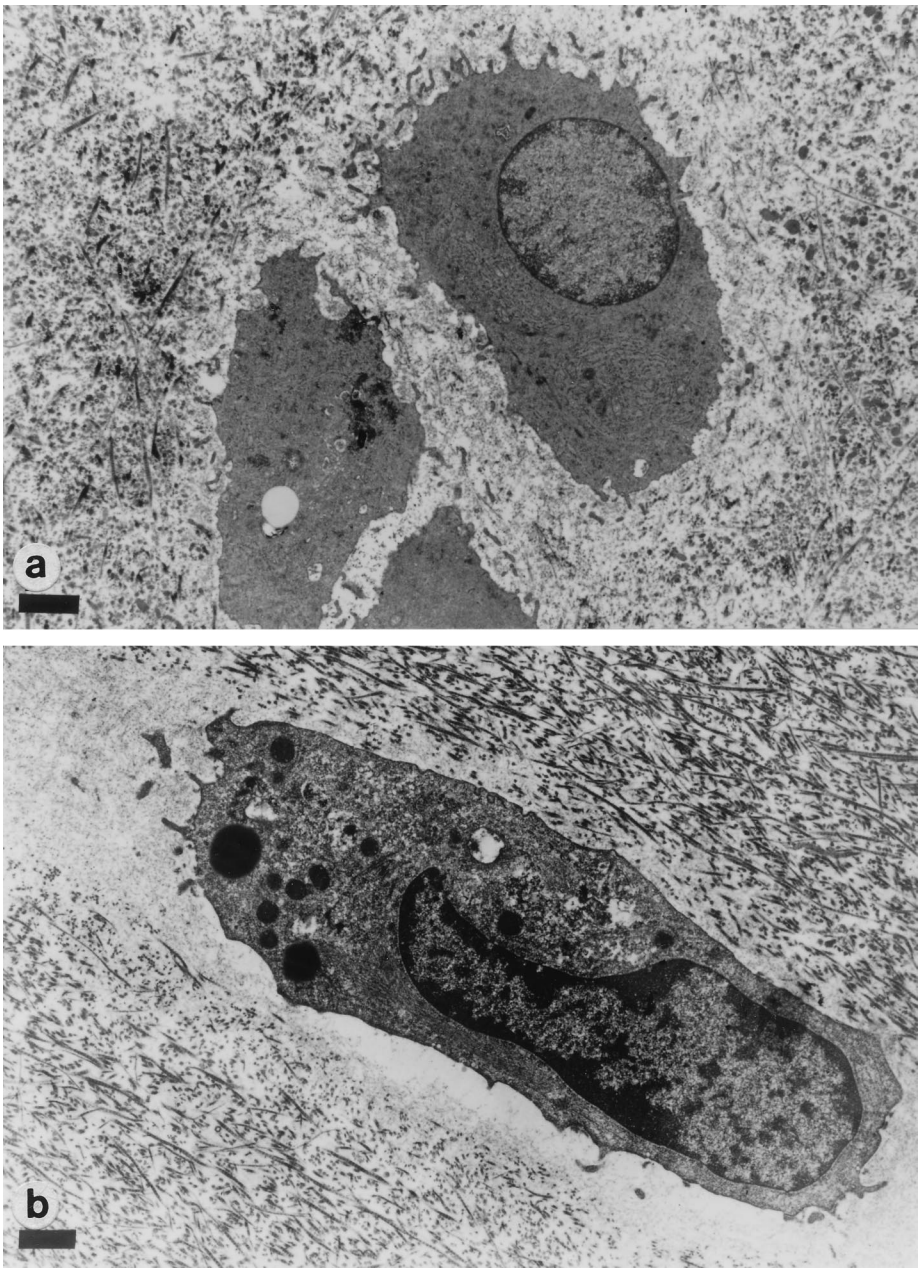

FIG. 1. Human cartilage explants harvested after 2 weeks of culture without quinolones show a slight metabolic activation. Bars, 6.66 μm.

tion of cartilage and clefts of the matrix originated from the empty chondrons (not shown).

Fluorocytometry after cell suspension culture in medium alone showed a significant increase in the rate of apoptosis of cells after 3 weeks of culture. The percentage of chondrocytes undergoing programmed cell death in medium alone was very low initially (1.1%; standard deviation [SD], 0.62), increased moderately at day 10 (3.4%; SD, 1.5), and after 3 weeks of in vitro culture rose to 11% (SD, 1.8). However, compared to results for incubation in medium alone, ofloxacin or ciprofloxacin had no effect on the induction of apoptosis.

Toxicity studies of juvenile animals showing arthropathic side effects of fluoroquinolones have led to a restricted use of these potent antibacterial compounds for children (3, 4, 9–12, 14, 24, 25, 29). However, the effectiveness against *Pseudomonas aeruginosa* and the convenience of oral administration of these drugs have recently augmented the rate of pediatric prescriptions. Subsequently, clinical safety profiles for children were found to be similar to those for adults (13, 17, 22, 27, 31). Radiographs and magnetic resonance imaging of the cartilage after long-term use of quinolones for children with cystic fibrosis did not show cartilage damage at the macroscopic signal resolution inherent in these methods (6, 18, 20, 21). Thus, it seems to be the consensus that quinolones can be used as safe and well-tolerated drugs, e.g., for maintenance antipseudomonal therapy in cystic fibrosis patients (22). We show toxic effects of quinolones on human adult chondrocytes, followed by obvious necrosis of the chondrocytes; these changes were not

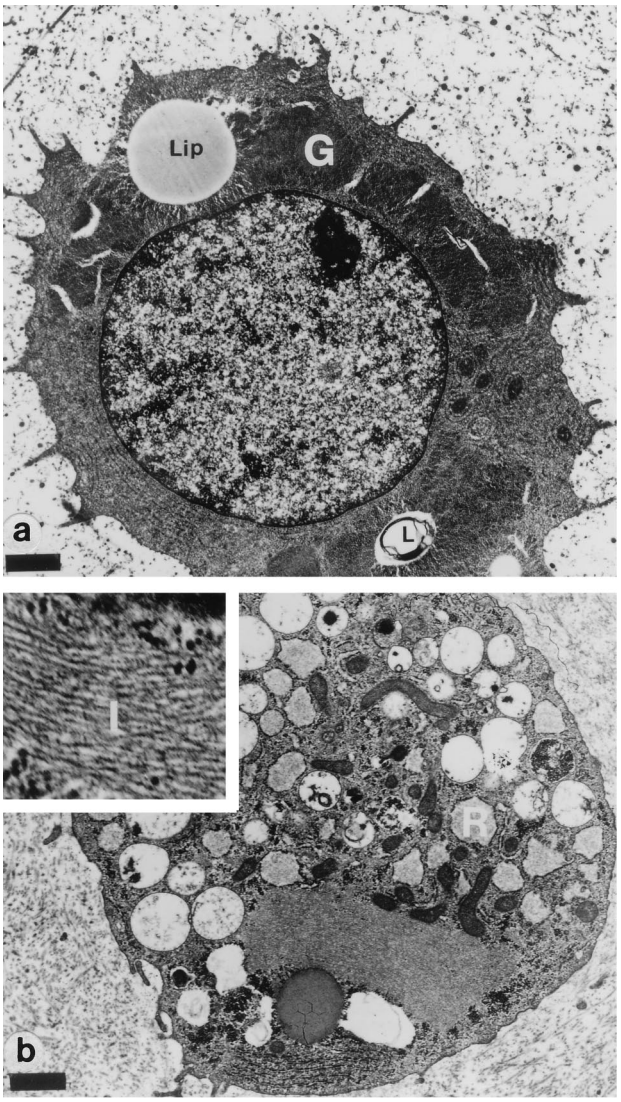

FIG. 2. Human cartilage explants after 2 weeks of incubation in culture medium containing 10 mg of ofloxacin per liter. (a) Chondrocyte of zone II containing large glycogen deposits (G) in the vicinity of a large lipid vesicle (Lip). A secondary lysosome (L) contains myelin-like degeneration products as an autophagocytotic phenomenon. Bar, 1.18 μm. (b) Degenerating chondrocyte with prominent perinuclear body consisting of intermediate filaments (inset; I) and dilation of the rough endoplasmic reticulum and intracytoplasmic vacuoles. Bar, 1.18 μm.

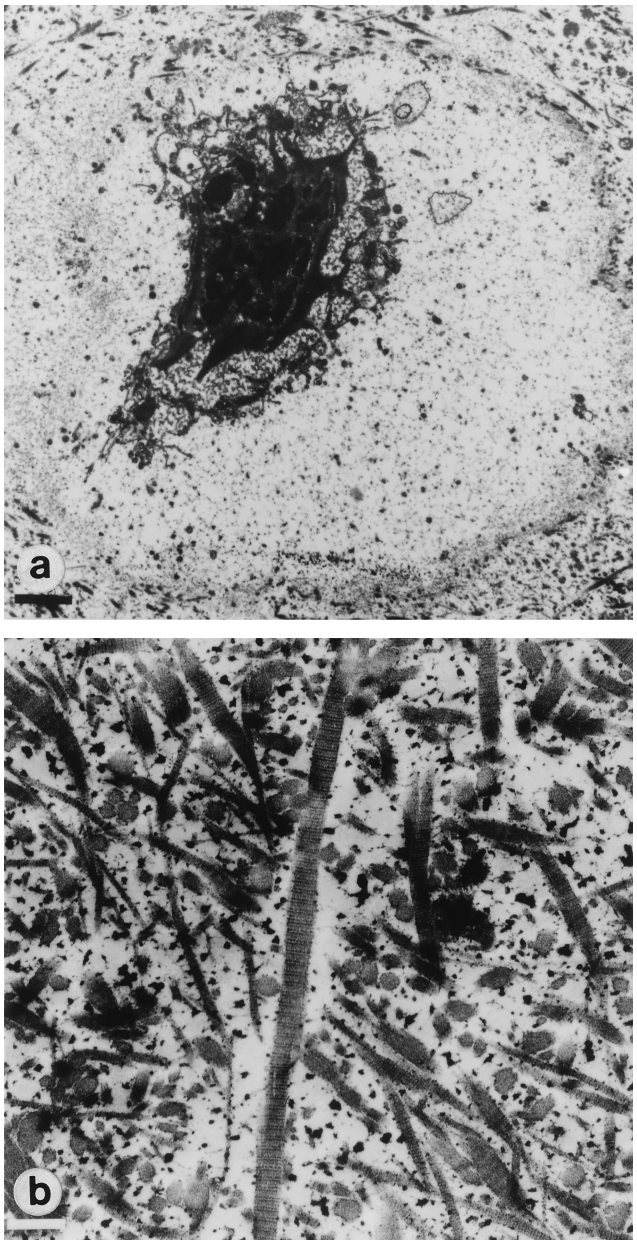

FIG. 3. Human cartilage exposed to 10 mg of ofloxacin per liter for 14 days. (a) Necrotic chondrocyte with disintegration of cytoplasm and condensation of nuclear chromatin. Bar, 1.18 μm. (b) Matrix with collagen fibrils, numerous proteoglycan particles, and some electron-dense calcium deposits. Bar, 0.27 μm.

seen in control cultures. Similar ultrastructural changes were reported for dogs in vivo as well as in vitro (3–5, 9, 11, 23). The concentration of the quinolones in the culture medium used in our study (1 mg/liter) corresponds to concentrations in serum of adult patients. We observed the ultrastructural changes after culture durations of 7 and 14 days for humans, while for beagle dogs, these changes had occurred already after 48 h of in vivo exposure (3), albeit with higher single doses of quinolones (300 mg/kg of body weight orally).

The molecular mechanisms blamed for quinolone chondrotoxicity include a deficiency of functionally available magnesium (7, 23, 26), inhibition of mitochondrial dehydrogenase and proteoglycan synthesis (11, 12), an altered metabolism of DNA (12, 16, 29) including inhibition of DNA polymerase (19), tissue accumulation of fluoride (18), and an increase of the respiratory burst in chondrocytes (10, 30). Nitric oxide-induced programmed cell death is an important mechanism in cartilage (2), and ciprofloxacin was reported to enhance production of the apoptogenic interleukin-1 (1). However, we could not detect an influence of quinolones on the rate of chondrocytes undergoing apoptosis.

The chondrotoxic effects of quinolone antimicrobial agents described here do not necessarily indicate clinically relevant cartilage damage in human adults. Nevertheless, an acceleration of (physiologic) cartilage degeneration cannot be excluded with the long-term application of these drugs. Moreover, the characteristic sensitivity of cartilage towards fluoroquinolones

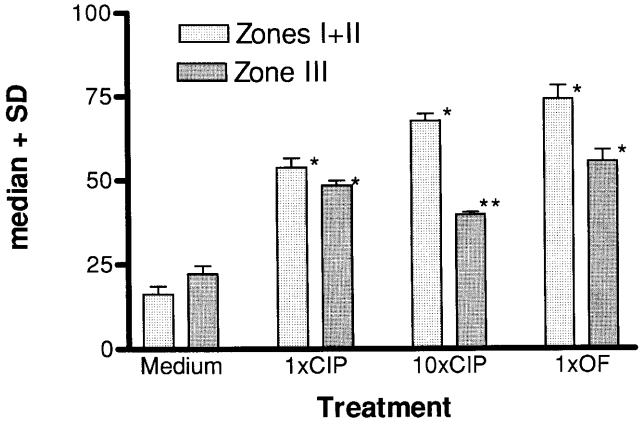

FIG. 4. Results of image analysis of light microscopy after 14 days of culture. The comparison of the percentages of necrotic and/or empty chondrons per 200 μm^2 yielded a significantly higher necrosis rate after quinolone exposure (*, $P < 0.001$; **, $P < 0.01$ versus medium alone; median of five different organ donors). Differences between the different quinolones used and their respective concentrations were not significant. CIP, ciprofloxacin; OF, ofloxacin.

may allow further insights into the tissue-specific metabolism of chondrocytes.

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